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Assessing the Nutritional Value and Suitability of Processed Rubber (*Hevea Brasiliensis*) Seed Meal for Rabbit Feed: A Focus on Anti-nutrient Degradation and Processing Methods

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Abstract

This study investigated the impact of processing methods on the nutritional value of rubber seeds. Two pretreatment techniques were explored to reduce anti-nutrients in the raw seeds: oven-drying the rubber seed meal for 30 to 180 minutes and soaking in an aqueous medium. The processed seeds were then collected and chemically analyzed, with samples taken from each treatment group. The proximate analysis of soaked rubber seed meal (SRSM) revealed the following composition: 2.80% moisture, 4.67% total ash, 18.70% crude protein, 8.34% crude fiber, 31.26% ether extract, and 34.23% carbohydrates. In contrast, oven-dried rubber seed meal (ODRSM) at 300°C for 30 minutes exhibited the highest values for moisture (3.92%), total ash (4.08%), crude protein (25.00%), crude fiber (8.16%), and ether extract (32.68%). Among the amino acids, glutamic acid, arginine, and cystine were the most abundant. Interestingly, oven-drying at 180 minutes proved most effective at removing anti-nutrients, with significant reductions in cyanide (6.53mg/g), tannins (0.051mg/g), saponins (0.1mg/g), and phenols (0.210mg/g) compared to soaking treatment. Based on these findings, the researchers recommend using rubber seeds heated to 300°C for 180 minutes as animal feed. This method results in low ash content, good protein levels, and a rich profile of macro and micronutrients. Overall, oven-drying appears to be a superior method for improving the nutritional value of rubber seed meal.

Keywords

Feed; pretreatment; proximate analysis; oven-drying; rubber seed; nutritive value; livestock

1. Introduction

Rubber is abundant in Thailand, Indonesia, Malaysia, Vietnam, Nigeria, Cote d'Ivoire, Liberia, and Brazil. Rubber plantations produce between 136 and 2000 kg/hectare of rubber seeds annually as a byproduct; however, only 25% are actually planted, while 75% end up as waste [1]. The rubber tree, *Hevea brasiliensis*, is a versatile, long-lasting plantation crop cultivated for its milky latex, the source of natural rubber. Each hectare of rubber trees yields 150-250 kg of seeds [2].

However, these seeds are often neglected and considered environmental waste in plantations. Rubber seeds can be a more cost-effective feed source compared to conventional concentrates like soybean meal, and crude palm oil which is imported and raises the overall feed cost [2, 3].

The shared use of crops like corn and soybeans for both human food and animal feed puts upward pressure on feed costs. This competition results in a shortage of traditional proteins and energy concentrates used in the production of animal feed. This issue has become a global concern and has prompted researchers and animal nutritionists to consider and evaluate the feeding value of non-conventional non-edible seeds that are abundant in the region [4]. Seeds classified as non-conventional are those that are not typically used in commercially produced rations or in animal feeding [5].

Several researchers have investigated the use of rubber seed meals as an alternative to conventional meals for domestic animals, reporting varying values of crude protein and ash content. Rubber seed meal offers higher essential nutrient values than many traditional seed meals due to its high concentration of easily digestible elements. Thus, it is an excellent substitute for protein and fat supplements in livestock diets [6]. Feed formulation can benefit from integrating rubber seed as a nutrient-dense meal. This option boasts a superior digestibility profile compared to many seed meals. Combined with its high essential nutrient content, it makes an excellent replacement for protein and fat supplements [7]. The quest is for a new, affordable, widely available protein and energy source for animal feed, reducing reliance on conventional crops used for human consumption. In addition, rubber seeds present a viable alternative. Monogastric animals are the main focus because ruminant animals are more susceptible to cyanide poisoning than non-ruminant animals [8].

However, the presence of cyanogen glycoside called Linamarin which can be enzymatically hydrolysed to release a toxin called Hydrogen Cyanide (HCN) has made rubber seed an unsuitable food source. Linamarin (Figure 1) is a cyanogenic glycoside found in rubber seed plant seeds [9]. Linamarin has the molecular formula ($C_{10}H_{17}NO_6$) and IUPAC name, Alpha-hydroxyisobutyonitrile-beta-D-glucopyranoside.

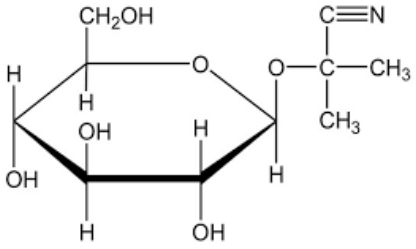

Figure 1. Molecular formula of Linamarin.

Apart from the high concentration of cyanide glycosides, there are other anti-nutritional elements that has prevented the proper use of the available nutrient. The additional anti-nutritional factors present in rubber seed include trypsin inhibitors, phytates, saponins, tannins, phenols, alkaloids, phytic acid, oxalate, and lectins. Significant losses can occur in animals due to anti-nutritional factors which have serious health consequences on the livestock such as decreased palatability, digestibility, and intoxication of various livestock classes and mortality of livestock [10].

Different biological, chemical, and physical methods can be used to detoxify the hydrocyanic acid, and processed rubber seed meal can be fed to livestock [11]. To ensure safe consumption, processing techniques are used to eliminate toxic elements like cyanogenic glycosides found in rubber seeds [12]. The processing techniques also improves the feeding quality of rubber seed, increasing its nutrient value, feed acceptability, and animal consumption. Building on existing research, this study explores the use of processed rubber seed meal as a sustainable alternative to conventional livestock feed for rabbits. By investigating the impact of oven drying durations and water soaking on anti-nutrient degradation, the research aims to identify the optimal processing method. Proximate analysis and amino acid profiling will be conducted on the processed meal to assess its nutritional value after eliminating harmful compounds.

2. Materials and Method

2.1 Materials and chemicals

Rubber seeds (*Hevea brasiliensis*) were sourced from the Rubber Research Institute of Nigeria in Edo State. Eight Hyla breed rabbits were acquired from a local livestock farm. All chemicals used were of analytical grade and employed without further purification. These included: Aqueous ethanol solution (C_2H_5OH) and Diethyl ether, Ferric chloride ($FeCl_3$), Potassium chloride (KCl), Sodium chloride (NaCl), Potassium permanganate solution ($KMnO_4$), Sodium carbonate solution (Na_2CO_3), Sodium hydroxide solution (NaOH), Hydrochloric acid (HCl), Folin ciocalteau reagent, and Gallic acid solution ($C_6H_2(OH)_3COOH$). TechnoPharmChem, MolyChem, and Sigma Aldrich provided the various chemicals.

2.2 Collection and preparation of rubber seed meal

Freshly harvested rubber seeds were divided into two equal portions for separate processing. Figures 2 and 3 illustrate the pictorial and schematic diagram outlining the production process for the rubber seed meals. In the oven-dried rubber seed meal (ODRSM), fresh rubber seeds were first dehulled by hand hammer. Then, they were milled into fine powder with a blender (Model BL330, Kenwood Blender, UK). The seeds were oven-dried (Vision Scientific Japan, Model LDO-210-E) at 300°C for varying durations: 30, 60, 90, 120, 150, and 180 minutes.

Figure 2. From seed to meal: See how raw rubber seeds become nutritious treats.

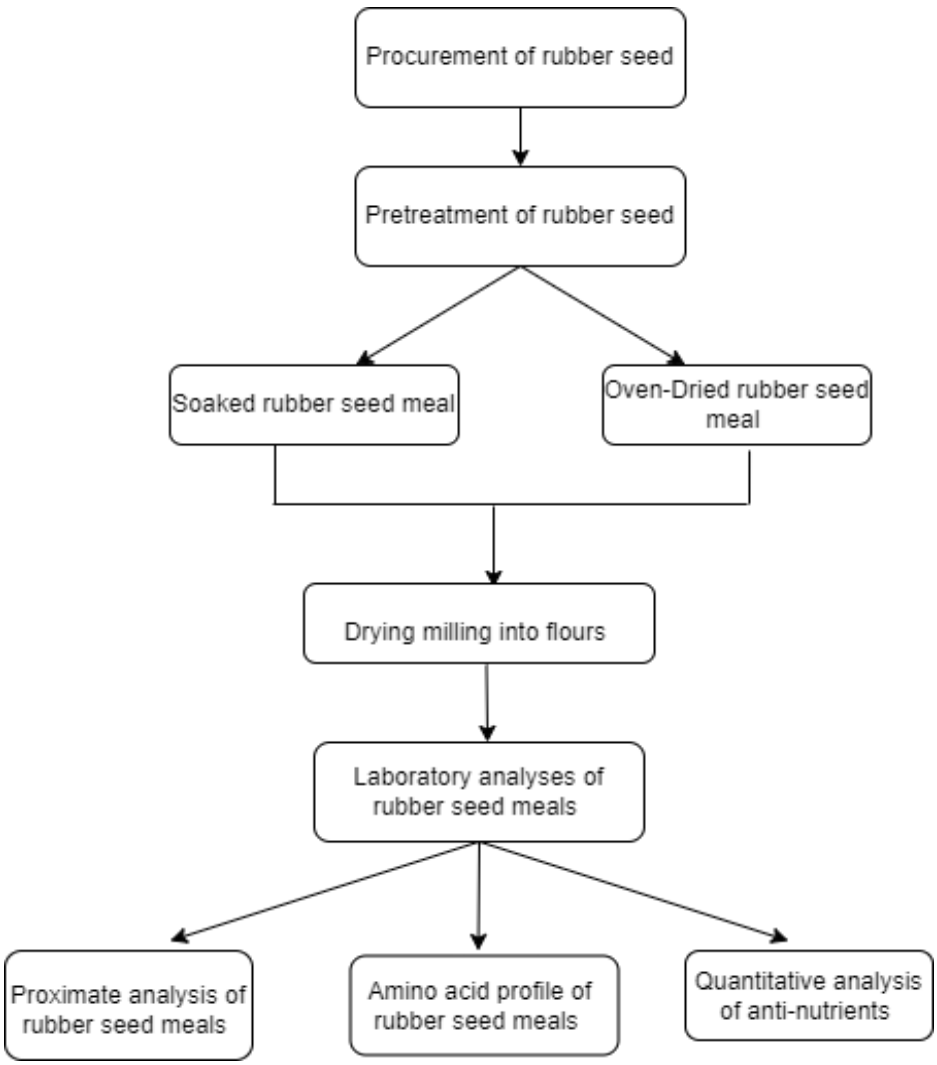

Figure 3. Flow diagram for the nutritional evaluation and degradation of rubber seed meals.

The soaked rubber seed meal, (SRSM), the seeds were dehulled by hand hammer. However, instead of drying, they were soaked in water for 3 days (72 hours) at a 1:3 seed-to-water ratio. The water was replaced daily. Afterward, the seeds were sun-dried for 3 consecutive days. Finally, they were ground into a fine meal using the same blender. Both processed meals (oven-dried and soaked) were stored in airtight containers with labels for further analysis.

2.3 Chemical analysis

The chemical composition of the samples was analyzed for moisture content (MC), dry matter (DM), ash content (AC), crude fiber (CF), crude protein (CP), ether extract (EE), and carbohydrate according to the Association of Official Analytical Chemist [15] methods in triplicate. An automated pre-column derivatization and online reversed-phase LC separation with a UF-Amino station for detection on a Shimadzu LCMS 2020 system was used to determine amino acids. The AmiNavi software (Shimadzu Corporation and Ajinomoto) was employed for data analysis and evaluation.

a) *Determination of moisture content*
The moisture content of the rubber seeds was determined using the gravimetric method outlined in the [16] guidelines. This method involves placing a sample in an evaporation dish and heating it in an oven at 105°C for 3 hours. After cooling the sample in a desiccator, its weight is measured. To ensure complete drying, the sample is reheated for another hour, cooled, and weighed again. The moisture content is then calculated as the percentage of weight lost during drying and reported as a percentage of the original sample weight. The moisture content was then calculated as follows:

$$moisture\ content(\%) = \frac{W2-W3}{W2-W1} \times \frac{100}{1} \quad (1)$$

where the initial weight of the petri dish, W_1, initial weight of the Petri dish after adding the sample, W_2 and final weight of the Petri dish after drying the samples, W_3 in grams.

b) *Determination of total ash content*
The ash content of the sample was measured using the furnace incineration gravimetric method [17]. A weighed amount of the sample (around 5 grams) was incinerated in a muffle furnace at 600°C until all organic matter burned off. The remaining ash, which matched the weight and color of the crucible, was weighed after cooling in a desiccator. The ash concentration was determined by subtracting the final weight from the initial weight and then dividing this difference by the initial sample weight.

$$Ash(\%) = \frac{W2-W1}{Weight\ of\ sample} \times \frac{100}{1} \quad (2)$$

W_1 represents the weight of the empty in grams, W_2 represents the weight of the crucible plus the ash in grams.

c) *Determination of fat content (Ether Extract)*
A 5 g sample was weighed and carefully wrapped in filter paper before being placed in a clean, dry, and cooled extraction thimble. To remove the fat, 200 mL of petroleum ether solvent was used. The mixture was heated for 30 minutes at 60°C in an oven after the top of the reflux flask was connected to a water condenser to remove any remaining solvent. The flask was then placed in a desiccator and weighed. The weight difference was used to calculate the oil (fat) extract weight, which was reported as a percentage of fat [14].

d) *Determination of crude protein*
This study followed the method outlined by [19], a sample of 0.5g was digested with 10 mL of concentrated H_2SO4 in a digestion flask, heated under a fume hood with a selenium catalyst tablet until a clear solution formed. The digest was then diluted to 100 mL in a volumetric flask. Next, 10 mL of the digest was mixed with 10 mL of 45% NaOH solution in a Kjeldahl distillation apparatus and distilled. The distillate was treated with a mixed indicator (bromo cresol green/methyl red) and collected in 50 mL of 40% boric acid. The distillate was then titrated with 0.02 N H_2SO4 until a color change from green to deep red occurred. A blank reagent was also digested, distilled, and titrated following the same procedure. Equation 3 is used to determine nitrogen content and, by extension, protein content.

$$N_2(\%) = \frac{100}{W} \times \frac{N\ X\ 14}{1000} \times \frac{Vt}{Va} \times T.B \quad (3)$$

1 mL of 1 N H_2SO_4 = 14 mg; Protein (%) = N_2 (%) × 6.25
W: weight of the sample analyzed (in grams); *N*: normality of H_2SO4 titrant (0.02 N); V_t: total volume of the digest (100 mL); V_a: volume of the digest distilled (10 mL); *T*: sample of titre value; *B*: blank titre value.

e) *Determination of crude fibre*
The method outlined in [18] was employed to quantify the fiber content. 5g of the processed sample were refluxed in 150 mL of 1.25% H_2SO_4 for 30 minutes. To remove any remaining particles, the boiled sample was thoroughly rinsed with

hot water using a double layer of fabric as a filter. The sample was then subjected to another 30-minute heating cycle in 150 mL of boiling NaOH solution. Following this, the sample was washed repeatedly with hot water, drained, and quantitatively transferred to a pre-weighed crucible. It was subsequently dried at 105°C until its weight remained constant. After drying and reweighing, the sample was ashed in a muffle furnace at 550°C for 2 hours and weighed once more after cooling. The difference in weight served to determine the fiber content, which was then expressed as a proportion of the total sample mass.

The crude fibre content of each sample was calculated gravimetrically by:

$$Crude\ Fibre(\%) = \frac{W2-W3}{Weight\ of\ sample} \times \frac{100}{1} \quad (4)$$

W_2 represents the weight of crucible and sample after washing, boiling and drying; W_3 is the weight of the crucible and sample of ash.

f) *Determination of carbohydrate*

Each sample extract (45 mL) was diluted with 450 mL of distilled water. Water and glucose served as controls (blank and standard, respectively). Separate test tubes were filled with the diluted filtrates. 5mL of freshly prepared 0.1% Anthrone reagent were added to each test tube, which were then stoppered and shaken gently to ensure thorough mixing. After labeling, the test tubes were placed in a rack and incubated in a 30°C water bath for 12 minutes. Following incubation, the tubes were removed and allowed to cool to room temperature. The absorbance of the samples and standard, compared to the blank, was measured at 630 nm using a spectrophotometer. The green color indicating the presence of glucose remained stable for approximately two hours**.** Equation determines the total amount of readily available carbohydrate. The total amount of readily available carbohydrate as a proportion of glucose using colorimetric assay

$$\text{Glucose (\%)} = \frac{(25 \times A)}{(X \times A_2)} \times \frac{100}{1} \quad (5)$$

where X is the weight of sample; A is the absorbance of the diluted sample; A_2 is the absorbance of diluted standard (glucose standard). Equation 5 relies on a calibration curve developed by [20] using a glucose standard. This curve assumes a linear relationship between absorbance and glucose concentration. The constant value of "25" originates from the specific steps involved in the assay protocol.

2.4 Anti-nutritional analysis

a) *Determination of Saponins*

5 g of each seed meal were weighed and placed in separate flasks. Each flask received a 20% ethanol solution (25 mL) and was shaken while being heated for 4 hours at 55°C in an incubator. The mixture was then filtered, yielding about 10 mL of liquid per sample. This liquid was transferred to a separating funnel where fats and pigments were removed using different solvents (diethyl ether and n-butanol). Finally, the desired extract was collected, and the remaining solvent was evaporated by heating the flask. The weight of the flask was measured before W_1 and after W_2 evaporation to determine the amount of extract obtained.

Finally, the remaining solution in the separating funnel was collected in a pre-weighed conical flask (W1). The flask was placed on a hotplate stirrer and heated until the solvent completely evaporated. After cooling, the final weight of the flask (W_2) was recorded.

$$\text{Total Saponins (mg/g)} = W_2 - W_1 \quad (6)$$

b) *Determination of Tannin*

The Folin-Denis colorimetric method, as described by [21, 22], was used to quantify tannin content. 5g of each sample were added to a 50 mL volumetric flask containing distilled water. 1mL aliquots of the extract were then transferred to separate tubes. To each sample aliquot, distilled water was added to make up the volume to 1 mL. A blank containing only 1 mL of water was prepared for comparison. Subsequently, 35% (Na_2CO_3) was added to each sample solution. Following a 5-minute incubation at room temperature, Folin's phenol reagent (diluted 1:2 with water) was introduced. Finally, the absorbance of each solution was measured at 640 nm using a spectrophotometer. A standard calibration curve constructed with gallic acid (1 mg/mL) served to determine the tannin content, expressed in milligrams per gram of extract (mg/g).

The tannin content was calculated using [13] model:

$$Tannin\ (\%) = \frac{100}{W} \times \frac{au}{as} \times \frac{Vt}{Va} \times C \quad (7)$$

where the weight of sample is "*W*" absorbance of test sample "*au*", absorbance of standard tanning solution "*as*", the total volume of extract, V_t, Volume of extract analysed, V_a, concentration of standard tannin Solution "*C*".

c) *Determination of phenol*

A method described by [20] was used to determine the total phenolic content (TPC) of rubber seed meal. This method utilizes a UV-VIS Spectrophotometer at 765 nm to measure the formation of a blue complex created by the reaction between phenolic compounds in the seed meal and a reducing agent. The absorbance reading is then compared to a standard curve generated using a gallic acid solution. This allows researchers to quantify the TPC and express it as milligram equivalents of gallic acid per gram of fresh rubber seed meal (mg GAE/g FW).

$$Phenol = \frac{C \times V}{W} \tag{8}$$

where C is the concentration of gallic acid calculated from the calibration curve (mg/ml), V is the volume of extract (mL), W is the weight of the sample (g).

3. Results and Discussion

3.1 Proximate analysis of processed rubber seed meals

Profiling of proximate parameters of rubber (*Hevea brasiliensis*) seeds revealed significant differences ($p<0.05$) in crude protein, moisture, crude fiber, ash, fat content (ether extract), and carbohydrates, as shown in Table 1. The study compared the effects of different treatments: oven-dried rubber seed meal (ODRSM) and soaked rubber seed meal (SRSM), with heating intervals of 30 minutes over 2 hours and soaking effects. The moisture content of OD (30 mins) and OD (60 mins) were highest at 3.92% and 3.88%, respectively, compared to OD (90-180 mins) values of 3.26%, 3.21%, 3.10%, and 2.88%. The lowest moisture content was 2.80% from soaked rubber seeds. These results align with previous studies by [3] reporting 3.99% and by [24] reporting 3%. Other studies, such as [25], found moisture contents of 5.8% in rubber seed meals.

Table 1. Nutrient Profile of rubber (*Hevea brasiliensis*) seed meal

	Percentage composition (%w/w)						
Proximate Composition	OD (30mins)	OD (60mins)	OD (90mins)	OD (120mins)	OD (150mins)	OD (180mins)	Soaked
MC	3.92	3.88	3.26	3.21	3.10	2.88	2.80
DM	96.08	96.12	96.74	96.79	96.90	97.12	97.20
AC	4.08	3.95	3.79	4.05	3.96	3.94	4.67
CP	25.00	23.10	21.20	22.90	24.30	23.60	18.70
CF	8.16	7.90	7.58	8.10	7.92	7.88	8.34
EE	32.68	31.10	30.82	31.80	30.78	29.64	31.26
CC	26.16	30.07	33.35	29.94	29.94	32.06	34.23

Notes. OD= Oven-Dried
MC=Moisture Content; DM=Dry Matter; AC= Ash Content; CF= Crude Fiber; CP= Crude Protein; EE= Ether Extract; CC=Carbohydrate Content.

The ash content of the rubber seed meals indicates the presence of inorganic elements in the samples. In this study, the ash content ranged from 3.79% to 4.67%. This result aligns with previous studies that reported ash content between 3.5% and 5.0% [24, 26]. However, it differs from studies that reported ash content of 2.6% [27] and 3.1% [3, 28, 29].

The crude protein (CP) results indicated a lower CP level in soaked rubber seed meal compared to oven-dried rubber seed meal. The oven-dried values ranged from 21.20% to 24.30%, which aligns well with previous findings of 22.3% [27] and 23.6% [30]. Soaking likely caused seed deterioration, leading to a lower CP of 18.70% and this is associated to leaching of nitrogenous compounds into the water [31, 32]. Nonetheless, this study on protein content supports previous observations that rubber seed is a viable protein source for livestock feed [3, 33].

The crude fiber content of oven-dried rubber seed meals (ODRSM) processed for 30 to 180 minutes varied slightly (7.88% -8.16%). This is within the range reported in other studies (7.41% - 17.41%, [3, 26, 34]. Soaked rubber seed meal (SRSM) had a slightly higher crude fiber content (8.34%) compared to oven-dried meals.

[5, 37, 38] investigated how consumption and digestibility are influenced by the presence of ether extract (crude fat). In this study, slight variations in the ether extract of rubber seed meals were found across the oven-dried seeds, ranging from 29.64% for OD (180 minutes) to 32.68% for OD (30 minutes). The soaked rubber seed meal also showed a slightly high value of 31.26%. The findings of 28.8% by [35] and 32.3% by [36] are consistent with the ether extract values

obtained in this study. This variation is suggested to be a result of the rubber seed meal preparation procedures or variations in the proportion of kernel content in the seeds, which could explain the high crude fat content.

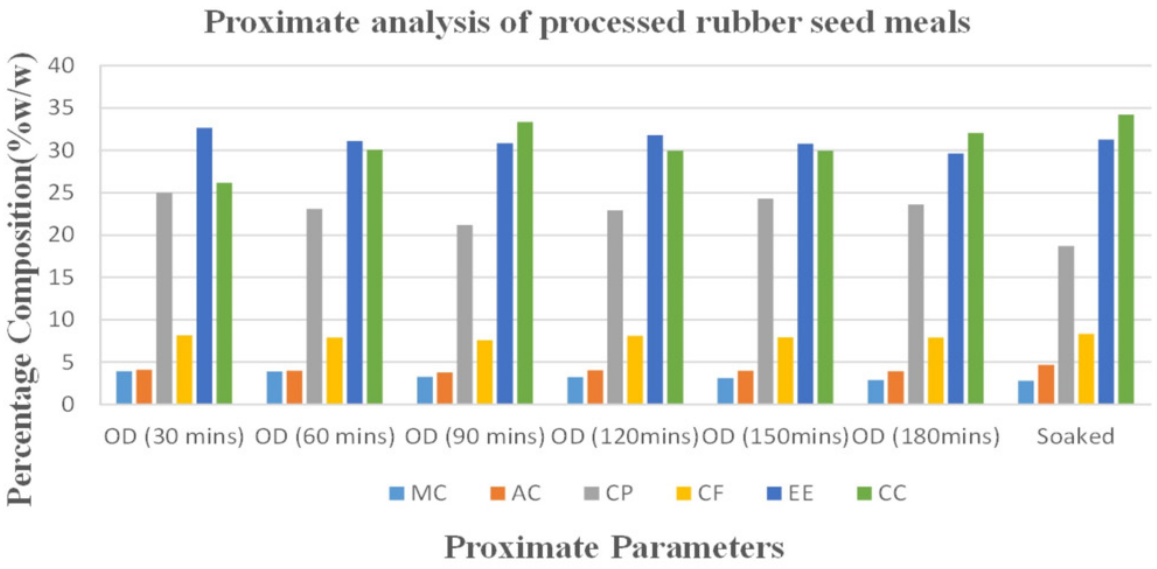

Figure 4. Proximate analysis of processed rubber seed for feed.

The carbohydrate content of the rubber seed meals exhibited a consistently high trend of values. Specifically, the soaked rubber seed meal recorded the highest carbohydrate content at 34.23%, while oven-dried rubber seed meals ranged from 29.94% to 32.06%. Studies by [39-41] revealed that animal diets enriched with seed meals containing high carbohydrate levels provide significant dietary energy.

Therefore, considering the carbohydrate content and studies by [42] on laboratory rats, [43] on pigs, and [6] on broiler chickens, rubber seed meal appears to be a promising partial or complete replacement for maize in animal and poultry diets.

3.2 Amino acid profile of processed rubber seed meals

The amino acid profiles of the processed rubber seed meals are detailed in Table 2 and Table 3. These compounds are crucial for supporting metabolic demands and maintaining optimal body composition and growth rates in livestock. Amino acids constitute the essential components of proteins, essential for sustaining life. Thus, adequate protein intake depends on the quantity and balance of amino acids in the diet. Variations in amino acid content were observed across the processed rubber seed meals. Amino acids are nutritionally classified as essential and nonessential for animals [40]. The extracted protein showed minimal amounts of essential amino acids, namely threonine, methionine, isoleucine, and phenylalanine.

Table 2. Essential Amino Acid (g/100g)

	OD (30mins)	OD (60mins)	OD (90mins)	OD (120mins)	OD (150mins)	OD (180mins)	Soaked
Threonine	2.55	2.54	2.48	2.52	2.60	1.50	3.49
Valine	5.49	5.50	5.55	3.20	5.45	1.80	5.56
Methionine	3.80	3.77	3.93	3.75	3.93	1.44	3.44
Lysine	6.70	6.44	6.63	6.59	6.60	9.60	6.94
Leucine	6.82	6.80	6.85	5.46	6.90	2.10	7.55
Isoleucine	3.30	3.55	3.40	2.95	3.03	1.40	2.15
Phenylalanine	2.23	2.15	2.04	1.44	2.07	1.55	1.60

Table 3. Non-Essential Amino Acid (g/100g)

	OD (30mins)	OD (60mins)	OD (90mins)	OD (120mins)	OD (150mins)	OD (180mins)	Soaked
Asparagine	8.55	7.10	6.60	6.55	6.55	6.77	11.20
Glutamic acid	17.23	17.90	19.20	15.20	19.23	13.22	23.80
Glycine	3.10	3.15	3.05	3.20	3.10	1.55	3.00
Histidine	2.30	2.33	2.40	2.22	2.30	2.50	3.11
Arginine	12.15	12.00	10.15	11.52	12.15	7.70	12.45
Proline	5.20	6.58	7.24	6.18	5.20	3.50	4.65
Serine	3.54	3.50	3.40	2.55	3.80	2.50	3.28
Cystine	13.90	13.75	13.60	13.55	13.60	10.26	15.30
Alanine	2.23	2.47	2.50	2.48	2.53	1.90	3.30
Tyrosine	1.30	1.33	1.45	1.40	1.45	1.13	1.10

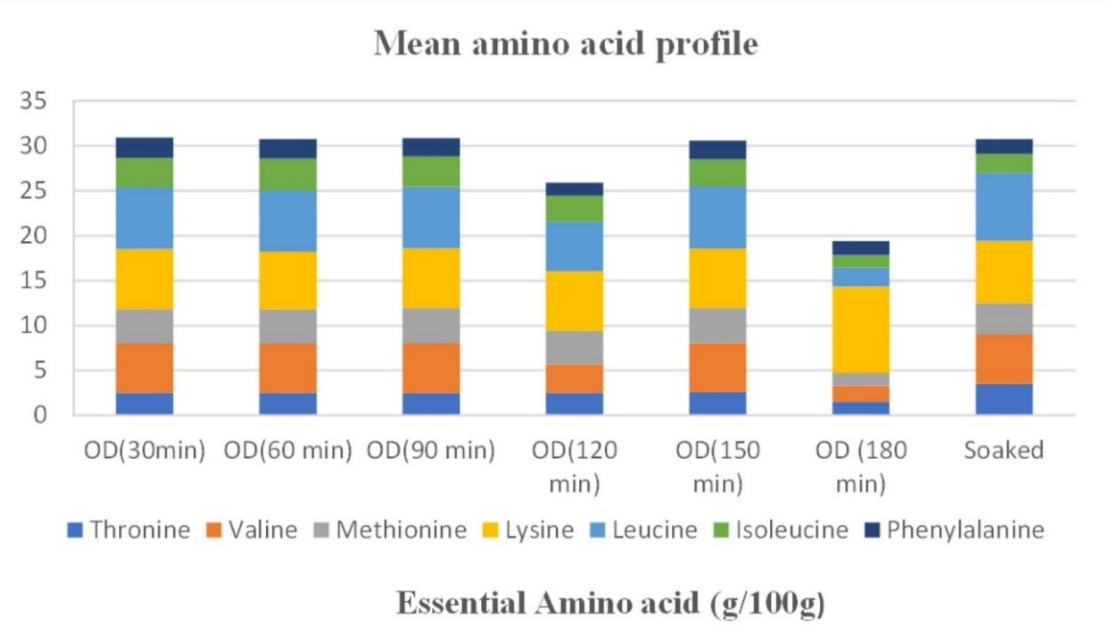

Figure 5. Average amino acid profile highlighting the essential amino acids in processed rubber seed meal.

The rubber seed meals are relatively low amount in some of the non-essential amino acids; histidine, glycine, serine, alanine, tyrosine and very high in some non-essential amino acids; glutamic acid, cystine, arginine, and asparagine. They are in moderate amount in proline, lysine, leucine, and valine respectively. Glutamic acid is considered a potentially necessary amino acid in some animals because it inhibits intestinal atrophy in certain circumstances [44].

Glutamic acid and cystine are the predominant amino acids in the processed rubber seed meal. Glutamic acid content reached 19.23g/100g% for OD (150 mins), the highest among the oven-dried rubber seed meals, and 23.80g/100g% for soaked rubber seed meal. Cystine content reached 13.90g/100g% for OD (30 mins) and 15.30g/100g% for soaked rubber seed meal. These findings align closely with results reported by [40] and [3], highlighting glutamic acid as a major amino acid in processed rubber seed. Both studies noted low levels of essential amino acids methionine and threonine. [3] reported a higher value of valine (7.08g/100g%) compared to (3.83g/100g%) as conveyed by [40]. Other essential amino acids were present in moderate amounts, while non-essential amino acids were not detailed in either study.

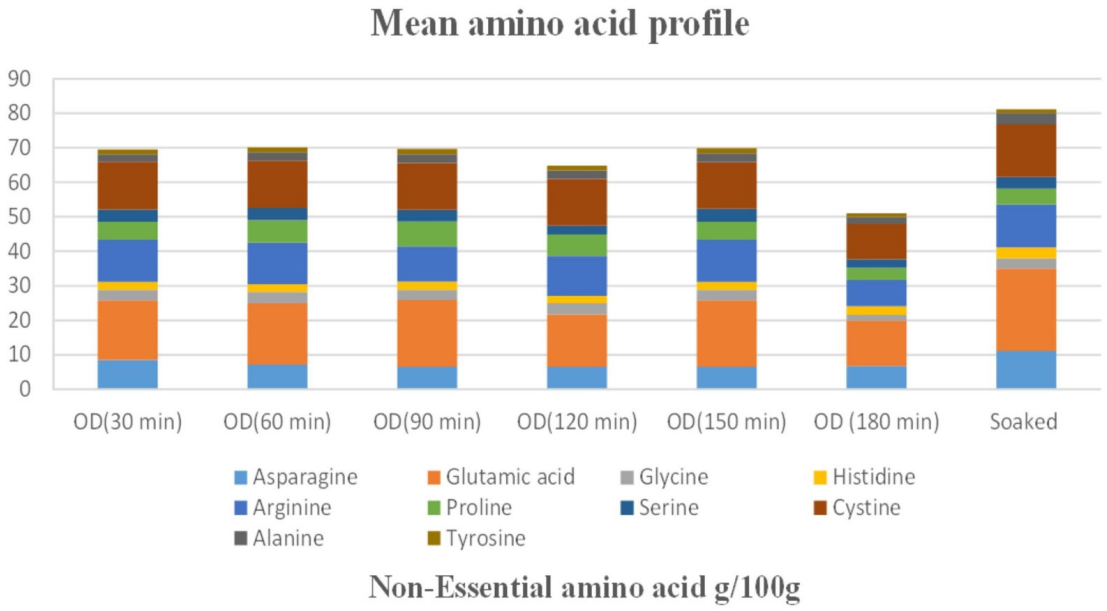

Figure 6. Mean amino acid profile showing non-essential amino acid of processed rubber seed meal.

3.3 Amino acid analysis of processed rubber seed feed

An automated pre-column d erivatization using an amino acid reagent for LC-MS, along with standard solutions, was performed using the Shimadzu LCMS 2020. This automation reduces human error and increases speed.

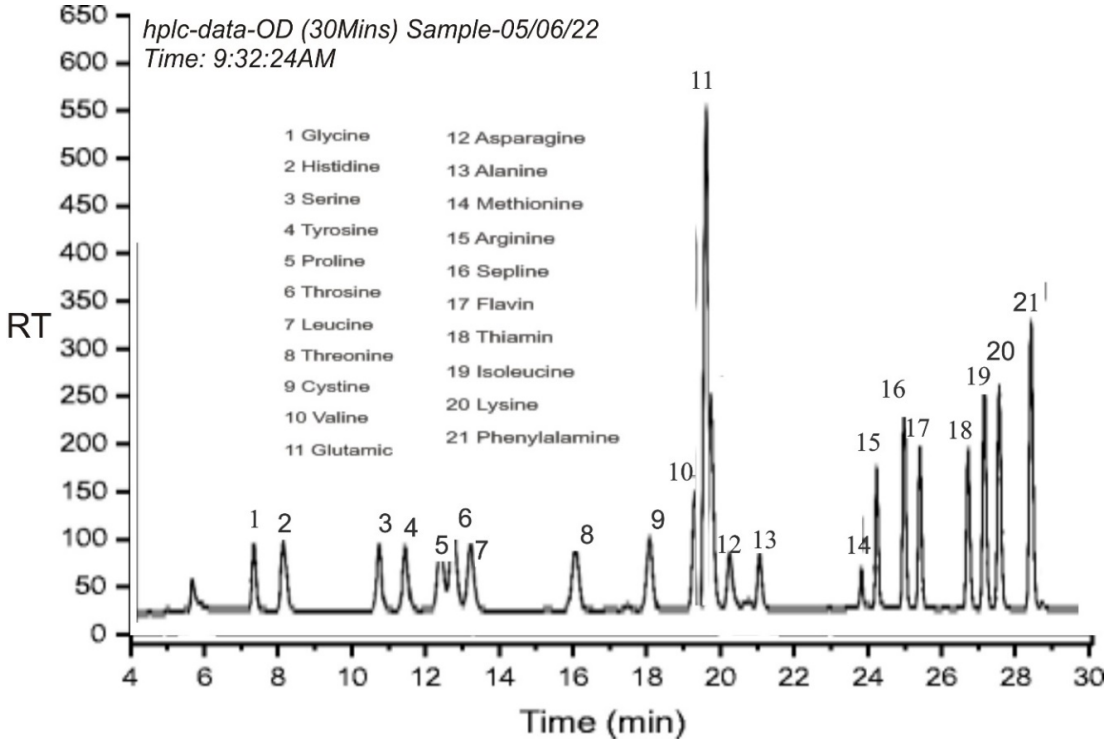

Figure 7. HPLC Data of OD (Oven-Dried) 30 minutes rubber seed meal.

Calibration standards at concentrations of 500 µmol/L, 300 µmol/L, 50 µmol/L, 10 µmol/L, and 2.5 µmol/L were prepared from a concentrated amino acids standard mixture. Seven processed rubber seed meals were used as test samples. Blank samples, calibration standards, and sample meals were all carefully prepared (after filtration). Internal standard solution (water for blanks) was added to deep well plate tubes along with the test probe, then filled to the top with acetonitrile (water for blanks). The amino acid derivatives were efficiently derivatized in the reaction unit at 60°C before

being transferred to the analytical column (2.1 × 100 mm, 2.0 µm Shimpack UF-Amino). The HPLC chromatogram plots the retention time (RT) against the detector response, showing the separation and identification of various amino acids in the sample.

Each peak in the chromatogram, as shown in Figures 7 and 8 for oven-dried samples at 30 and 180minutes, corresponds to a specific amino acid. Figure 9 illustrates the chromatogram for the soaked seed meal. Glutamic acid (peak 11) shows the highest intensity in the oven-dried rubber seed meal compared to the soaked seed meal, indicating it is the most abundant amino acid in this sample. Methionine (peak 14) and threonine (peak 6) show lower intensities, indicating lower concentrations in the sample. This HPLC data at 180 minutes reveals the presence and quantity of different amino acids in the oven-dried rubber seed meal, with glutamic acid and being the most abundant.

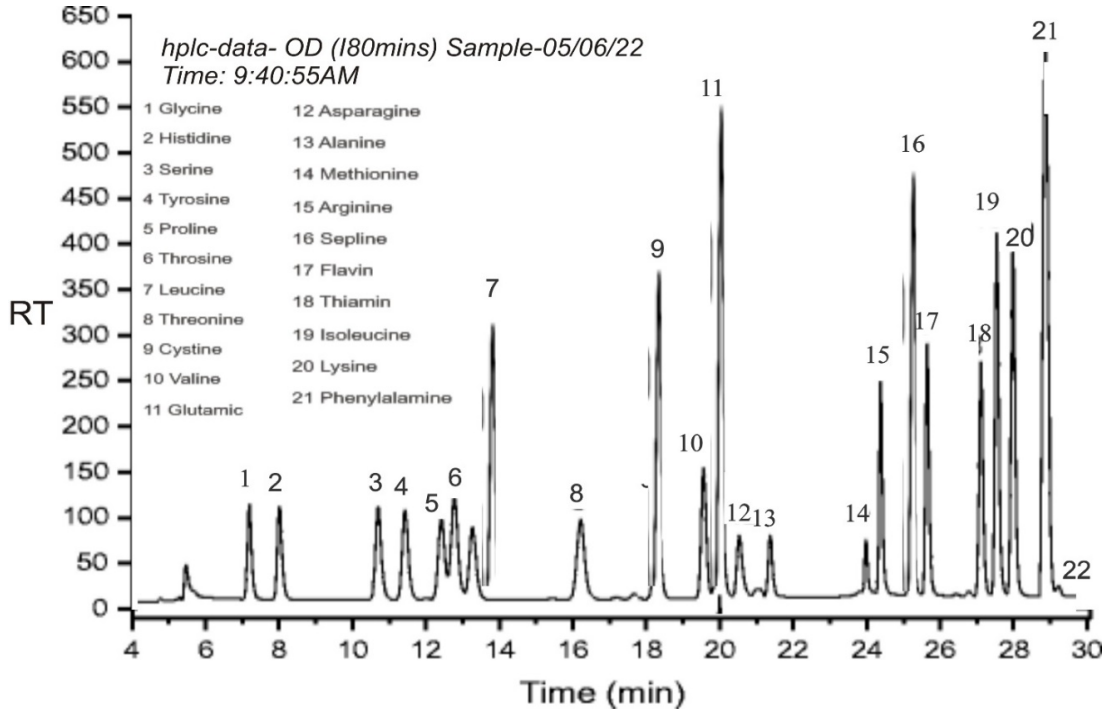

Figure 8. HPLC Data of OD (Oven-Dried) 180 minutes rubber seed meal.

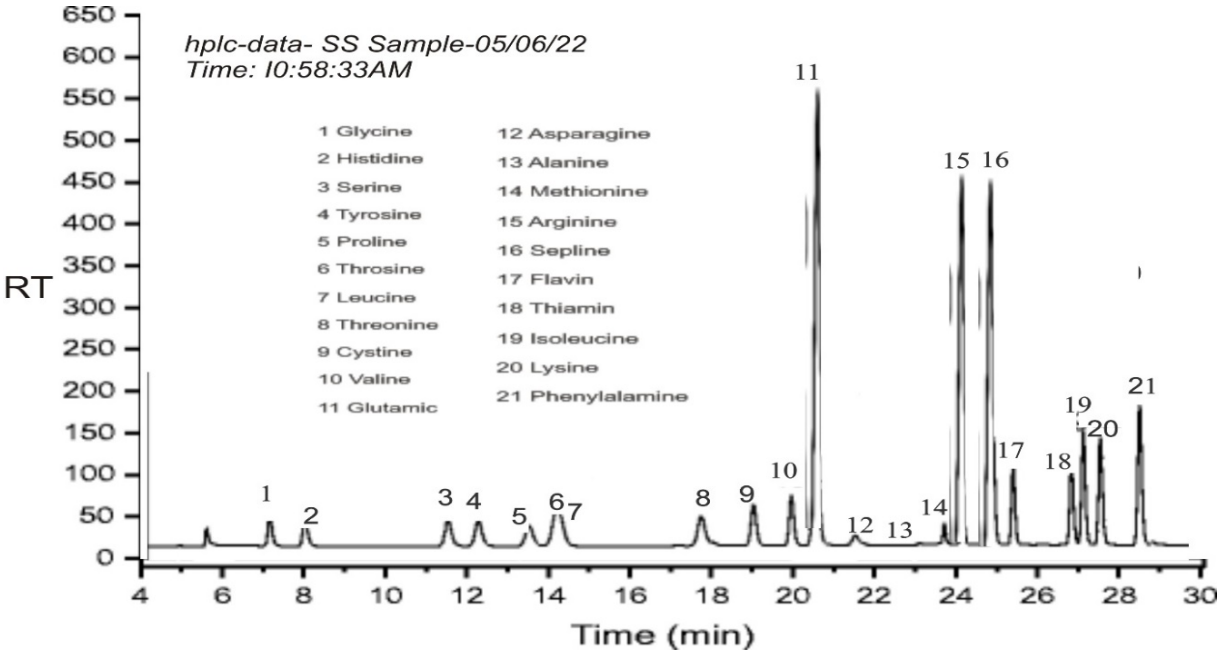

Figure 9. HPLC Data of SS (Soaked Seed) rubber seed meal.

3.4 Quantitative analysis of phytochemicals

Previous studies by [5, 45-47] reported the presence of anti-nutritional and bioactive chemicals in rubber seed kernels. This finding is further supported by the phytochemical analyses of our processed rubber seed meals under various treatment conditions, as shown in Tables 4 and 5.

Table 4. Cyanide removal in rubber seed meal under various treatment conditions at 300 ºC

rubber seed meal	cyanide removal (mg/g)
Raw	137.01
30	1.02
60	1.53
90	2.01
120	3.21
150	5.78
180	6.53
Soaked	2.06

Table 5. Phytochemical content of rubber seed meal under various treatment conditions

Phytochemicals (mg/g)	Raw	OD (30mins)	OD (60mins)	OD (90mins)	OD (120mins)	OD (150mins)	OD (180mins)	Soaked
Tannins	8.98	0.056	0.076	0.103	0.028	0.061	0.051	0.116
Saponins	4.99	0.7	0.6	0.5	0.4	0.2	0.1	0.2
Phenols	2.77	0.183	0.221	0.217	0.132	0.103	0.210	0.179

Rubber seed meal contains harmful cyanogens. In this study, the cyanogen content in raw seed meal was measured at 137.01 mg/g. Other studies have reported higher cyanide contents in raw rubber seed meal: [5] reported 249 mg/g, [6] stated 315 mg/g, and [12] conveyed 415.10 mg/g. In comparison, the result from [48], at 138 mg/g, aligns closely with the findings of this study.

The results in Table 4 indicate that a substantial amount of cyanide can be removed from rubber seed meals through oven-drying for varying periods rather than soaking. Cyanide removal consistently increased with longer drying times. The lowest cyanide removal was observed at OD (30 minutes) while the highest removal occurred at OD (180 minutes).

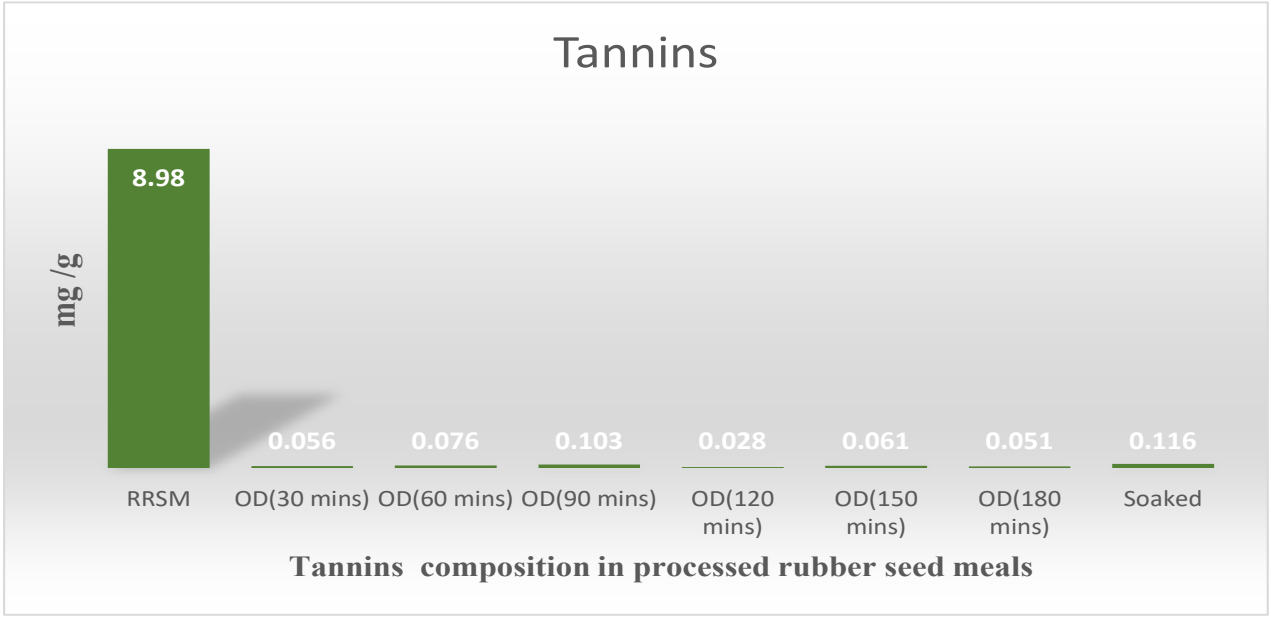

Figure 10. Tannin composition in processed rubber seed meal.

The results in Table 5 indicate a reduction in other anti-nutrients, specifically tannins, saponins, and phenols, which were initially present in relatively high concentrations. Other anti-nutrients were also present but at relatively low concentrations, well below the safety limits for animal consumption. The differences in the levels of these phytochemicals were statistically significant at $p<0.05$.

In Figures 10 and 11, there was a significant reduction across all rubber seed meals as oven drying time increased, tannins are the primary antioxidants because they scavenge free radicals. The rubber seed had a tannic acid content of 8.98 mg/g. with the lowest tannin content observed at 120 minutes of oven drying. Soaked rubber seed meal exhibited high tannin content. For saponins the lowest value was recorded at 180 minutes of oven drying, clearly indicating that longer oven drying times make the rubber seed meal safer for animal consumption, as levels were well below safety limits. This prolonged heating effect also reduced the saponin content in the raw rubber seed.

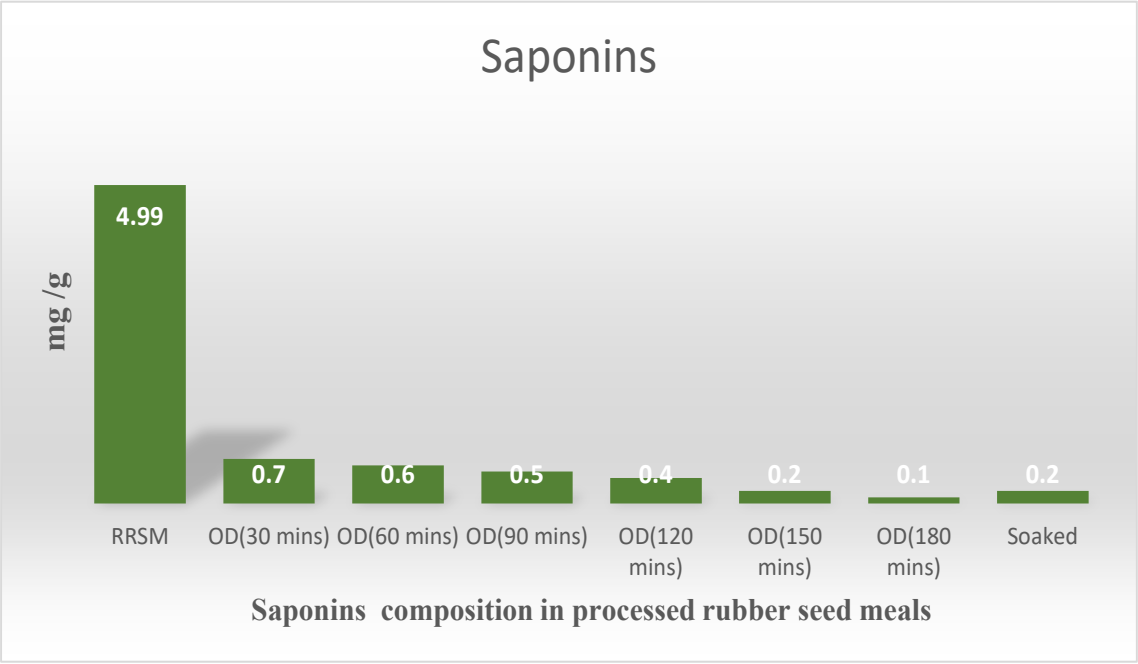

Figure 11. Saponins composition in processed rubber seed meal.

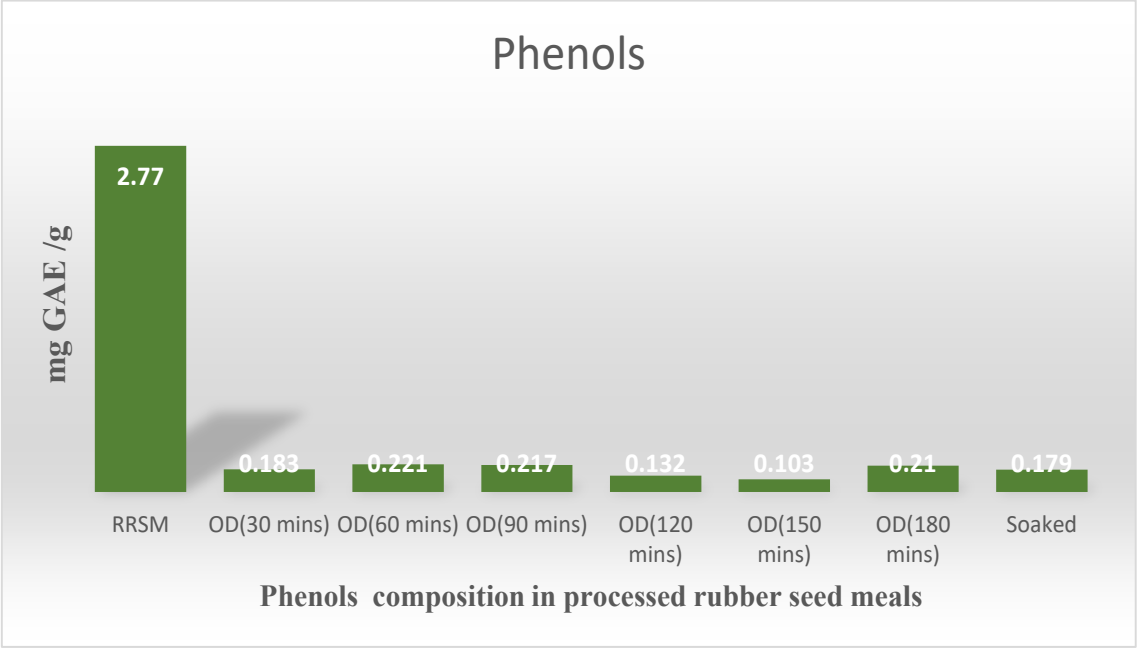

Figure 12. Phenol composition in processed rubber seed meal.

In Figure 12, Phenolic compounds, including phenolic acids and flavonoids, are the primary antioxidant constituents of natural plant products. These compounds effectively terminate radicals by donating a hydrogen atom, thereby blocking the initial step in lipid oxidation. The raw rubber seed has a phenolic concentration of 2.77 mg/g. After 150 minutes of oven drying, the phenolic content was at its lowest value. The effect of heating is evident in the reduction of phenol levels in the raw rubber seed.

4. Conclusion

This study demonstrates that processed rubber (*Hevea brasiliensis*) seed meal is a promising alternative feed ingredient for rabbits. The oven-drying method, especially at 300°C for 180 minutes, significantly reduces anti-nutritional factors, including cyanide, tannins, saponins, and phenols, to safe levels while preserving a favorable nutrient profile. Compared to soaked seed meals, oven-dried seed meals yielded higher crude protein, ether extract, and balanced amino acid profiles, particularly in essential and non-essential amino acids such as glutamic acid, arginine, and cystine. Furthermore, the carbohydrate-rich composition of the processed seeds enhances their energy value. These findings validate oven-drying as an effective processing method to enhance the nutritional value of rubber seed meals while ensuring safety for animal consumption. Future research should explore the long-term health impacts and economic viability of incorporating processed rubber seed meal into commercial livestock feed formulations.

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